Comparison of Homogenization Methods for Recovering *Salmonella* Enteritidis from Eggs

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ABSTRACT

For *Salmonella* Enteritidis (SE) detection, shell eggs have been homogenized with stomachers, with electric blenders, and by hand massaging. However, to date, there have been no published reports addressing whether the method of homogenization affects the recovery of SE from raw eggs. Three inoculum levels (10, 126, and 256 SE cells per pool of 10 eggs) were used to conduct three experiments. The 10-egg pools were homogenized by one of four homogenization methods—mechanical stomaching, electric blending, hand massaging, and hand stirring—for 30 s. The homogenized eggs were then incubated at 37°C, and SE colonies were enumerated after 24 and 48 h of incubation. After 24 h of incubation, no SE was recovered from egg samples from stomached or electrically blended pools inoculated with <10 cells, while levels of 10^6 CFU/ml were found for samples from whipped or hand-massaged pools inoculated with <10 cells. Similarly, after 24 h of incubation, the numbers of SE cells recovered from hand-massaged or hand-stirred egg pools inoculated with 126 cells were significantly larger than the numbers recovered from stomached or electrically blended egg pools inoculated with 126 cells. The number of SE cells recovered from samples homogenized with a blender was still significantly smaller than the numbers recovered from samples homogenized by the other three methods when the inoculum level was increased to 256 CFU per pool. However, the SE count for all samples approached 9 $\log_{10}$ CFU/ml after 48 h of incubation. It is concluded that the detection of small SE populations in shell egg samples could be improved with the use hand massaging and hand stirring for homogenization.

The high proportion of human salmonellosis outbreaks that are attributable to *Salmonella* Enteritidis (SE) remains a major concern for the commercial layer industry, which strives to control SE in eggs and egg products. The egg industry is required to test flocks for SE by testing egg samples and subjecting eggs from known SE-positive flocks to pasteurization according to the U.S. Department of Agriculture's proposed rules *(15)*. The low contamination rate and the antimicrobial effect of egg albumen have contributed to the difficulty in detecting SE in raw eggs. The primary site of contamination in eggs would seem to be either the albumen or the outside of the vitelline membrane, although the egg yolk has been also identified as a potential site for contamination *(3, 9)*. The growth rate of SE in the egg yolk is generally very high because of the yolk's high iron content, while the growth in egg albumen is inhibited because of the antimicrobial and iron-restricting compounds in albumen *(11, 13, 14)*. However, SE cells cannot initially make use of the iron found in the yolk because the vitelline membrane of the fresh egg does not permit either the ingress of bacteria into the yolk's contents or the release of iron into the albumen. Therefore, the homogenization of egg contents is an essential step in the isolation SE from the egg's contents.

The pooling and homogenization of 10 to 30 eggs has been suggested to increase the detection rate and the effectiveness of the SE isolation procedures *(2)*. Direct plating of egg contents on a selective medium has been recommended to expedite detection procedures and minimize costs *(15)*. The effectiveness of supplementation of egg contents with iron or concentrated broth media after homogenization to enhance SE growth has been investigated for direct plating *(1, 5)*. Homogenization for the detection of SE in eggs has been carried out with stomachers, with electric blenders, and by hand massaging in various studies *(1, 2, 8)*. The mixing of egg contents with a sterile spoon or with some other sterile instrument is recommended for *Bacteriological Analytical Manual* methods *(16)*. However, to date, the effect of the homogenization method on the growth of SE in liquid whole egg has never been reported. The objectives of this study were to compare the extents of SE multiplication in liquid whole egg pools homogenized by four different methods—mechanical stomaching, electric blending, hand massaging, and hand stirring—and to determine the optimum homogenization method for the isolation of SE from raw eggs.

MATERIALS AND METHODS

Preparation of SE culture. A mutant strain of SE phage type 13a (SERR), resistant to 0.1 mg of rifampicin (Sigma Chemical Co., St. Louis, Mo.) per ml, was isolated as previously described *(12)* and used as a marker organism. A loopful of SERR was transferred from a nutrient agar plate containing 0.1 mg of rifampicin per ml to tryptic soy broth containing 0.1 mg of rifampicin per ml and incubated for 24 h at 37°C. The overnight culture

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TABLE 1. *Populations of* Salmonella *Enteritidis (SE) recovered from egg homogenates originally inoculated with 10 cells per pool and prepared by four different homogenization methods*

Incubation time (h)	SE plate count ($\log_{10}$ CFU/ml) for homogenization method[a]:			
	Hand massaging	Hand stirring	Stomaching	Electric blending
24	6.5 ± 2.6 (100)	5.9 ± 2.2 (100)	ND[b]	ND
48	8.6 ± 0.7 (100) A	8.7 ± 0.3 (100) A	6.5 ± 2.1 (75) B	ND

[a] Mean ± standard deviation; the percentage of samples ($n = 4$) testing positive for SE is shown in parentheses. Means with different letters in the same row are significantly different ($P < 0.05$).

[b] ND, not detectable.

was diluted in 0.1% buffered peptone water (pH 7.2) to yield SERR concentrations of 10^0 to 10^9 CFU/ml and plated on nutrient agar and on brilliant green agar supplemented with 0.1 mg of rifampicin per ml (BGAR; Difco Laboratories, Detroit, Mich.) for enumeration.

Eggs. Eggs were purchased from a local grocery store in the Washington, D.C., area. Any dirty eggs or eggs with chipped, cracked, or broken shells were removed and discarded. The remaining eggs were stored at 5°C until they were used. Eggs were surface disinfected by soaking them in a solution consisting of 3 parts 70% alcohol and 1 part 10% Lugol's iodine solution *(7)*. After soaking, eggs were allowed to air dry.

Preparation of inoculated eggs. Egg contents were inoculated with SERR with a modified version of a previously described method *(6)*. The air-dried eggs were aseptically broken, and then the yolk and albumen were separated into individual sterile 200-ml plastic beakers. For the inoculation of a single separated yolk, 0.1 ml of SERR was evenly sprayed with a pipet onto the intact surface of the yolk membrane. The inoculated yolk was held at room temperature for 5 min, and then the albumen from a single egg was poured into the sterile beaker containing the inoculated yolk. Four pools of nine eggs for each homogenization method in each experiment were prepared by aseptically cracking nine eggs into sterile stomacher bags (Tekmar Company, Cincinnati, Ohio). One SERR-contaminated egg was then mixed into each pool to yield a final pool size of 10 eggs. Three experiments were conducted with different inoculum levels: 10, 126, and 256 cells per pool of 10 eggs. Two uninoculated pools of 10 eggs were included with each treatment group as negative controls.

Sample homogenization. Four pools per inoculum level were stomached for 30 s with a Stomacher 400 lab blender (Seward Laboratory, London, UK) at normal speed (200 rpm). Four pools per inoculum level were homogenized in sterile stomacher bags (Tekmar Company) by hand massaging for 30 s to ensure the complete mixing of albumen and egg yolk material. Four pools per inoculum level were aseptically poured into a 1,000-ml sterile beaker, and then egg yolks and egg whites were thoroughly mixed with a sterile spoon for 30 s. The mixed egg contents were transferred back into the stomacher bag for incubation. Four pools per inoculum level were aseptically poured into a sterile blender jar and blended for 30 s at normal speed (Model 31BL91, Waring Commercial Laboratory, New Hartford, Conn.). The blended egg contents were transferred back into the stomacher bag for incubation.

Enumeration of SE in egg pools. Four pools of 10 eggs were prepared for each treatment. All egg samples in stomacher bags were tightly sealed with a sterile rubber band and incubated at 37°C for 48 h. At 24 and 48 h, samples were taken for SERR detection by adding 1 ml from each egg pool to 9 ml of 0.1%

buffered peptone water, with subsequent brief vigorous vortexing. For SERR enumeration in egg contents, serial dilutions in buffered peptone water were carried out, and the dilutions were plated on BGAR in duplicate and incubated at 37°C for 48 h before colonies were counted. Suspected SE colonies were randomly picked and confirmed biochemically and serologically as described previously *(12)*.

Statistical analysis. The numbers of SE cells found in the homogenized egg contents were transformed to $\log_{10}$ counts and analyzed statistically by one-way analysis of variance with GraphPad (San Diego, Calif.) software. Differences among treatments were examined for significance with Tukey's procedure.

RESULTS AND DISCUSSION

A preliminary experiment showed that the time (30 or 60 s) for which eggs were subjected to homogenization did not significantly affect recovery. The effects of different homogenization methods on SE growth in the egg pools inoculated with 10 SE CFU are shown in Table 1. After 24 h of incubation at 37°C, the SE populations recovered from samples prepared by manual means (hand massaging or hand stirring) were ca. 10^6 CFU/ml. However, no SE was detected for samples prepared by mechanical means (stomaching or electric blending) after 24 h of incubation. After a further 24 h of incubation, the SE population for samples prepared by hand massaging or hand stirring was approximately 3.9×10^8 CFU/ml, whereas the SE population for positive stomached samples (75%) was 3.1×10^6 CFU/ml after 48 h of incubation. No SE growth was observed for samples homogenized with an electric blender. Gast and Holt *(5)* reported that only 43% of egg pools tested positive for SE when 100 ml of egg contents was inoculated with 10 CFU, homogenized with a stomacher, and incubated for 24 h at 37°C. The present results, based on a lower inoculum level, are consistent with that previous report. Since the concentration of SE in raw eggs is normally very low, the homogenization method used in the initial sample preparation could be critical for the efficacy of an SE detection procedure.

The results presented in Table 2 show that for pools initially inoculated with 126 SE cells, samples that had been mixed manually had significantly larger populations of SE after 24 h of incubation than did samples that had been mixed mechanically. The lowest levels of SE were recovered from egg contents mixed with an electrical blender. After 48 h of incubation, SE levels for samples homogenized by manual methods were still higher (by ~1 $\log_{10}$

TABLE 2. *Populations of* Salmonella *Enteritidis (SE) recovered from egg homogenates originally inoculated with 126 cells per pool and prepared by four different homogenization methods*

Incubation time (h)	SE plate count ($\log_{10}$ CFU/ml) for homogenization method[a]:			
	Hand massaging	Hand stirring	Stomaching	Electric blending
24	8.2 ± 0.2 A	8.5 ± 0.7 A	3.0 ± 3.0 B	1.6 ± 0.7 B
48	9.3 ± 0.1 A	9.3 ± 0.2 A	8.4 ± 0.6 A	8.4 ± 0.4 A

[a] Mean $\pm$ standard deviation ($n = 4$). Means with different letters in the same row are significantly different ($P < 0.05$).

CFU/ml) than those for samples homogenized by mechanical methods.

When the inoculum level was increased to 256 CFU per pool, SE populations increased to ≥ 9.5 log CFU/ml for samples homogenized by manual methods, whereas the growth of SE was slower in samples homogenized by stomaching and by electric blending (7.6 and 5.0 $\log_{10}$ CFU/ml, respectively) (Table 3). The SE levels for samples homogenized with the blender was statistically lower than those for samples homogenized by manual methods ($P < 0.05$). However, the SE populations reached ~9 $\log_{10}$ CFU/ml for all samples after 48 h of incubation, indicating that even when the initial inoculum is relatively high, the homogenized eggs should be incubated for >48 h when the direct plating method is used for detection.

In all three experiments, whether the initial inoculum level was low or high, the numbers of SE cells recovered from eggs homogenized by mechanical means, notably by electric blending, were smaller than the numbers recovered from eggs homogenized by manual means. This finding may indicate that antimicrobial proteins in albumen mix with the egg yolk more homogeneously when mechanical means are used and are therefore more available to inhibit SE growth for a certain period. Furthermore, it is not clear whether physical stress from the electrical blender (such as shear stress and heat) or the antimicrobial components of eggs caused the death or injury of SE in samples prepared with the electrical blender.

The results of previous studies indicate that the minimum number of SE cells per pool required for 100% detection sensitivity is 10^7, which is equivalent to approximately 10,000 cells per ml of egg contents, assuming that a pool of 10 eggs consists of approximately 500 to 600 ml *(4)*. Therefore, the multiplication of SE to concentrations consistently detectable without further enrichment is critically important when the direct plating method is used as a rapid and practical means for the detection of SE in eggs. The detectability of SE in eggs is limited by the infrequent presence of SE in individual eggs, the very small populations of SE in SE-positive eggs, and the inhibitors that are naturally present in egg albumen.

Previous studies on the detection of SE in eggs have focused primarily on the development efficient sampling procedures, including the pooling of eggs, the incubation of eggs at room temperature for extended periods, and the supplementation of the enrichment broth with iron. The methods recommended by regulatory agencies often include preenrichment and selective enrichment steps to improve the detection rate. It is clear that the annual cost of mandatory screening of eggs from table egg breeder flocks, and perhaps from table egg production flocks, with the currently recommended test methods would be substantially high for producers. A survey of the nation's largest egg producers, representing over 100,000,000 layers, indicated that these producers prefer cost-effective techniques for the control of SE *(10)*. Therefore, a rapid and cost-effective method for the detection of SE in raw eggs is essential for the egg industry.

Chen et al. *(1)* reported that percentage of SE detected by the direct plating method in raw egg contents supplemented with 0.5 mg of $FeSO_4$ per g was significantly higher than the percentage detected in raw egg contents without iron supplementation. However, 63.3% of Chen et al.'s unsupplemented egg samples yielded SE, and 90% of their supplemented samples yielded SE when low levels of SE (2 CFU per egg) and electric blending were used. These authors concluded that the low concentration was attributable to incomplete detection. Gast *(2)* also reported that direct plating allowed SE detection for only 47.1% of the inoculated egg pools incubated for 4 days at 25°C when low inoculum concentrations (<10 CFU per pool) and stomaching (for 15 s) were used. However, both studies failed to show the effects of homogenization methods on the detection of SE in egg contents. The current study clearly shows that the homogenization method used is a critical factor in the rapid detection of SE in eggs.

TABLE 3. *Populations of* Salmonella *Enteritidis (SE) recovered from egg homogenates originally inoculated with 256 cells per pool and prepared by four different homogenization methods*

Incubation time (h)	SE plate count ($\log_{10}$ CFU/ml) for homogenization method[a]:			
	Hand massaging	Hand stirring	Stomaching	Electric blending
24	9.7 ± 0.4 A	9.5 ± 0.3 A	7.6 ± 1.1 AB	5.0 ± 2.4 B
48	9.6 ± 0.2 A	9.7 ± 0.6 A	9.6 ± 0.3 A	8.8 ± 0.5 A

[a] Mean $\pm$ standard deviation ($n = 4$). Means with different letters in the same row are significantly different ($P < 0.05$).

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